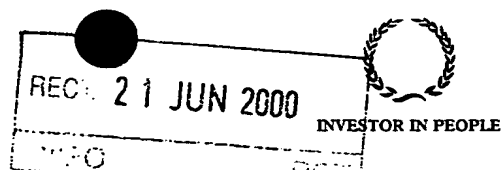




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4

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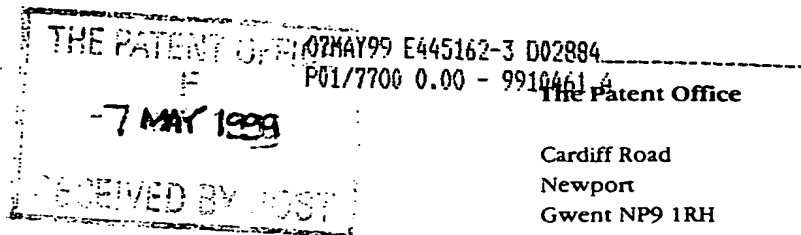
Dated

- 9 JUN 2000

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Request for grant of a patent

(See the notes on the back of this form. You can also get an explanatory leaflet from the Patent Office to help you fill in this form)



1. Your reference	P23670/RWA/GST/RMC		
2. Patent application number (The Patent Office will fill in this part)	07 MAY 1999	9910461.4	
3. Full name, address and postcode of the or of each applicant (underline all surnames)	Ewos Limited Technology Centre Unit 1 Kingsthorpe Park Houston Industrial Estate Livingston EH54 5DB United Kingdom		
Patents ADP number (if you know it)			
If the applicant is a corporate body, give the country/state of its incorporation	76 351 39001 RdeS		
4. Title of the invention	"Pigment"		
5. Name of your agent (if you have one)	Murgitroyd & Company		
"Address for service" in the United Kingdom to which all correspondence should be sent (including the postcode)	373 Scotland Street Glasgow G5 8QA United Kingdom		
Patents ADP number (if you know it)	1198013 ✓		
6. If you are declaring priority from one or more earlier patent applications, give the country and the date of filing of the or of each of these earlier applications and (if you know it) the or each application number	Country	Priority application number (if you know it)	Date of filing (day / month / year)
7. If this application is divided or otherwise derived from an earlier UK application, give the number and the filing date of the earlier application	Number of earlier application		Date of filing (day / month / year)
8. Is a statement of inventorship and of right to grant of a patent required in support of this request? (Answer 'Yes' if: a) any applicant named in part 3 is not an inventor, or b) there is an inventor who is not named as an applicant, or c) any named applicant is a corporate body. See note (d))	Yes		

Patents Form 1/77

9. Enter the number of sheets for any of the following items you are filing with this form.
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Continuation sheets of this form	-
Description	6
Claim(s)	-
Abstract	-
Drawing(s)	-

10. If you are also filing any of the following, state how many against each item.

Priority documents	-
Translations of priority documents	-
Statement of inventorship and right to grant of a patent (<i>Patents Form 7/77</i>)	-
Request for preliminary examination and search (<i>Patents Form 9/77</i>)	-
Request for substantive examination (<i>Patents Form 10/77</i>)	-
Any other documents (please specify)	-

11. I/We request the grant of a patent on the basis of this application.

Signature Murgitroyd & Company Date 6 May 1999
MURGITROYD & COMPANY

12. Name and daytime telephone number of person to contact in the United Kingdom

Roisin McNally 0141 307 8400

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Notes

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1 **PIGMENT**

2
3 This invention relates to a method of improving
4 pigmentation in fish flesh. Most specifically the
5 invention discloses a method whereby through the
6 addition of cholesterol there is an enhanced uptake of
7 pigment.

8
9 An increase in market competition, coupled with the
10 widespread availability of fish in supermarkets has led
11 to an increase in the demand put on the quality of fish
12 products.

13
14 Current mass production of salmonides such as salmon
15 and trout is required to meet current consumer demand
16 which exceeds that which can be met by fish produced in
17 a natural, wild environment.

18
19 There are variations though between the fish produced
20 naturally and those which are specifically farmed to
21 meet consumer demand. One such difference is a
22 variation in the colour of flesh between the two types.

23
24 It is considered by the consumer that a stronger red
25 colour of the flesh which is seen in the natural, wild

1 fish is more desirable and has a greater aesthetic
2 appeal than the paler red coloured flesh of farmed
3 fish.

4
5 In an effort to achieve the flesh colour
6 characteristics of the wild fish, pigments are added to
7 the feed given to farmed fish with the intent that the
8 uptake, by ingestion of the pigment, will lead to a
9 subsequent change in the colour of the flesh.

10
11 Preferably pigments are selected which will lead to a
12 change in this red colour and examples of such pigments
13 are canthaxanthin, astaxanthin, zeaxanthin, beta-
14 carotene and similar.

15
16 Such processes are not limited to fish, as the
17 modification of the colour of a naturally produced
18 foodstuff is a current trend. The aesthetic appeal of
19 the product is thought to be enhanced to the customer
20 through modification of the feed ingredients to
21 influence the characteristics of the final product, in
22 particular the colour.

23
24 An example of another such process currently known in
25 the art is the alteration of the feed ingredients given
26 to chickens and hens, such that the colour of the yolk
27 of the eggs that are produced is modified from that of
28 the natural colour. The result of this process is that
29 the product has an increased aesthetic appeal and as
30 such leads to a greater desirability for consumer
31 consumption.

32
33 The principles employed in the manipulation of the
34 colour of egg yolk are the basis for the change in fish
35 flesh colour. It is desirable for the flesh of the
36 fish to be altered to any specific requirement which

1 may be set. One such method of altering the fish flesh
2 colour would be through the introduction of pigments
3 into the diet.

4

5 The basis for this would be that generally the flesh is
6 constructed from substrates in the dietary intake.

7

8 This document suggests that the incorporation of a
9 pigment into the diet, either in combination with the
10 foodstuffs directly, or as a separate entity introduced
11 into the diet such that it will enter the same
12 metabolic pathways as other ingested and absorbed
13 nutrients will also end up as a constituent of the
14 flesh.

15

16 The pigment will lead to a change in the colour of the
17 flesh into which it is incorporated.

18

19 The incorporation of the pigment into the flesh may not
20 be efficient and this document identifies a method of
21 enhancing such pigment uptake.

22

23 The benefits of a method by which the uptake of pigment
24 by the fish is enhanced are wide-ranging and cover both
25 biological and economical aspects.

26

27 The addition of pigments such as canthaxanthin can have
28 a drastic economical effect on the cost of producing
29 fish feed pellets, due to the expensive cost of
30 pigments such as canthaxanthin. As such a more
31 efficient mechanism of producing the effects of
32 canthaxanthin will lead to a reduction in the amount
33 that needs to be added to the feed initially.

34

35 The reduction in amount of pigment or other similar
36 synthetic compounds will also have biological effects.

1 as the fish will be ingesting a reduced amount of the
2 synthetic pigment additive.

3

4

5 Investigations have been carried out, with the aim of
6 identifying substances which enhance the uptake of
7 pigments which may be added to the feed to improve
8 pigmentation.

9

10 Cholesterol was used as one of the enhancers due to its
11 properties as an auxiliary agent in uptake. Cholesterol
12 is an important lipid in some membranes and the plasma
13 membranes of eukaryotic cells are usually rich in
14 cholesterol, this steroid also modulates the fluidity
15 of eukaryotic membranes. Due to these properties
16 cholesterol was identified as a substance with the
17 potential to enhance pigment uptake.

18

19 It is an object of the present invention to provide a
20 method facilitating the enhanced uptake of pigment
21 through the addition of cholesterol.

22

23 According to the present invention there is provided, a
24 method to enhance uptake of pigment by fish, the method
25 comprising feeding fish with cholesterol and pigment.

26

27 Preferably the cholesterol and/or pigment will be in
28 the fish feed.

29

30 Also preferably the cholesterol will be provided in the
31 same medium as the pigment.

32

33 Preferably, the pigment will lead to a change in flesh
34 colour of fish.

35

36 The invention also provides fish feed comprising



1 cholesterol and pigment.

2

3 The invention also provides the use of cholesterol to
4 enhance uptake of pigment to fish flesh.

5

6 An experiment showing how the incorporation of
7 cholesterol can enhance pigment uptake is described
8 below.

9

10 Investigations of Enhance Pigment Uptake in ATS (Trail
11 9866).

12

13 Fish of mean weight 120g, were fed for a period of 72h
14 on one of two diets;

15

16 Diet 1: contains approximately 40ppm of
17 canthaxanthin (Cx).

18

19 Diet 2: contains 40ppm canthaxanthin (Cx) plus
20 0.48% (total feed, 3% in lipid coating phase) of
21 cholesterol. Cx and cholesterol were added in the
22 coating.

23

24 Results for the treatments can be seen on Table 1.

25

26 It can be clearly seen from the results shown in Table
27 1 that the fish fed with cholesterol in feed (Diet 2)
28 shown almost a 50% increase in the plasma Cx level
29 compared to the control feed. Additionally this trend
30 is repeated in both replications of the experiments.

31

32 Further experimentation is currently being carried out
33 looking directly at pigment deposition. Also
34 experiments investigating the level of astaxanthin
35 uptake with or without the incorporation and addition
36 of cholesterol will also be investigated.

TABLE 1

Replicate	Feed No.	Treatment	Level % feed	Feed Cx mg/kg	Plasma Cx μ g/ml mean (STD)
1	1092	CR		40.51	0.94 (0.5)
2					0.64 (0.4)
1	821	CR + Cholesterol	0.48	45.67	1.42 (0.57)
2					1.45 (0.96)

CR = carophyll red